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Complementary Hypoglycemic and Anti-Hyperglycemic Activity of Various Extracts of Fenugreek Seeds in Rats

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Abstract: In present study, five extracts of fenugreek seed with different solvents (water, ethanol, methanol, hexane and chloroform) alone and in combination with glimepiride were tested for hypoglycemic and anti-hyperglycemic activity in rats by screening blood glucose for 6 h. Water extract exhibited highest hypoglycemic and anti-hyperglycemic activity in rats among all the extracts, while hexane and other extracts exhibited least and moderate activity, respectively. Water extract was further studied to dose dependent [200, 100 and 50 mg kg^{-1} body weight (b.wt.)] hypoglycemic and anti-hyperglycemic effects alone and in combination with glimepiride (20, 10 and 5 mg kg^{-1} b.wt.). The combination of water extract (200 mg kg^{-1} b.wt.) and lower dose of glimepiride (5 mg kg^{-1} b.wt.) has shown safer and potent hypoglycemic as well as anti-hyperglycemic activity and not created severe hypoglycemia in normal rats, while higher doses (200 mg kg^{-1} b.wt. of water extract and 10 and 20 mg kg^{-1} b.wt. of glimepiride) were generated lethal hypoglycemia in normal rats. The results of present study enforced to say that, the water extract of fenugreek seeds has higher hypoglycemic and anti-hyperglycemic potential and may use as a complementary medicine to treat the diabetic hyperglycemia by significantly reducing dose of standard drugs.

Key words: Fenugreek, hypoglycemic, diabetes, herbal, glimepiride

INTRODUCTION

Diabetes mellitus is a global health problem and considered as a major factor of the immature morbidity and mortality, worldwide (King *et al*., 1998). It is defined as a group of disorders characterized by deficient insulin secretion or peripheral insulin resistance resulting in hyperglycemia and develop life threatening complications such as atherosclerosis, nephropathy, neuropathy and retinopathy (Clark, 1994). Till date, no permanent therapy has been developed for complete cure of diabetes and its complications. Various pharmacological agents involved medication to control blood glucose levels; some of them have undesirable side effects (Rowden and Fasano, 2007). Consequently, there is an urgent need to search some strong and safer antidiabetic components from natural sources.

Because of potential threat to quality of life and the chronic nature of diabetes many people turn to Complementary Therapies (CT) to assist them to cope and control the disease and eventually reduce the complications (Israili *et al*., 2007). The main CTs used for diabetes are nutritional, spiritual, herbal etc. More than 400 traditional Indian plants are reported to antidiabetic potential, only a small

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number of these have received scientific and medical evaluation to assess their efficacy (Grover *et al*., 2002; Saxena and Vikram, 2004). Among these *Trigonella foenum graecum* (Fenugreek) is a good example of folk medicines. The consumption of fenugreek by diabetic patient is common practice in India (Srinivasan, 2005). The hypoglycemic potential of fenugreek has been extensively reviewed (Brasch *et al*., 2003; Madar and Stark, 2002). Similarly, Petit *et al*. (1993, 1995) were demonstrated that hypoglycemic potential of fenugreek seeds are because of steroid saponins. Recently, Hannan *et al*. (2007) find that, the soluble dietary fibre fraction of fenugreek have hypoglycemic potential in type 1 and 2 diabetic rats by delaying carbohydrate digestion and absorption and enhancing insulin action. However, after various experimental proofs the most active extract of fenugreek seeds and its interaction with standard drugs is not still clear, which further need to study with a well designed experiment. This study was conducted to identify most active extract of fenugreek seeds and its dose dependent hypoglycemic and anti-hyperglycemic potential in rats. It has been speculated that, if most active extract of fenugreek seed may reduce the dose of standard drugs i.e. glimepiride (a member of sulphonylureas, commonly used as an insulin secretor), which may give safer treatment strategy by replacing synthetic drugs with natural regimens.

MATERIALS AND METHODS

Preparation of Extracts

Seed of *Trigonella foenum graecum* was purchased from Gwalior (India) market and air dried and powdered with grinder. The powdered plant seed material was extracted with polar solvents like ethanol, methanol and water and non polar solvents i.e., n-hexane and chloroform by using Sox-halet method.

Determination of Phyto-Constituents

The chemical composition of total phenols, alkaloids, tannins, saponins and flavonoids in different extracts were determined by methods described elsewhere (James and Thewlis, 1952; Edeoga *et al*., 2005). In brief, tannins were recorded by reacting with 0.1% ferric chloride solution, saponins were measured by mixing with olive oil and flavinoids were estimated with 1% aluminium solution.

Animals and Experimental Schedule

Male Wistar rats of 4-6 week old 121-129 g body weight (b.wt.) were housed in polypropylene cages at 22±3°C ambient temperature and 55±5% humidity in 12/12 light and dark cycle. This study was completed in two phases: Phase 1: The best hypoglycemic extract was selected by orally injecting 200 mg kg^{-1} b.wt. dose of each extract in 12 h fasted normal animals. To study anti-hyperglycemic activity, 2 g kg^{-1} b.wt. (20% solution) dose of glucose was administered to 12 h fasted animals at the same time of extract ingestion. Phase 2: Water extract was found to the most active hypoglycemic among other extracts. Three different doses of water extract (200, 100 and 50 mg kg^{-1} b.wt.) and glimepiride (20, 10 and 5 mg kg^{-1} b.wt.) were tested for hypoglycemic and anti-hyperglcaemic potential in both normal as well as glucose over-loaded rats.

Estimations of Blood Glucose

After oral administration of plant extracts and/or standard drug, the blood samples were collected from tail tip and glucose was monitored using glucometer strips (Roche Diagnostics, Indiana, USA) at 1 h interval for 6 h.

Statistical Analysis

Data were represented as Means±SD of 6 animals in each group and analysis of variance was performed by using SPSS (SPSS Inc. Chicago). The significant difference among groups were analysed with the help of student's t-test. The values with $p<0.05$ were considered statistically significant.

RESULTS

Phytoconstituents in Seed Extracts

Alkaloid and flavonoid contents were significantly higher in methanol, hexane and chloroform than those of water and ethanol extracts. However, tannins and saponins were significantly higher in water extracts than others, while no significant differences were observed in phenol contents among all the extracts (Table 1).

Hypoglycemic Activity of Various Seed Extracts

The results of Table 2 indicate that the oral administration of water extract of fenugreek seeds reduced 41% blood glucose in normal rats after 2 h. Ethanol extract of fenugreek seeds also reduced blood glucose by 25% after 2 h in the normal rats, while methanol extracts was moderately reduced it (13%) after 2 h. No significant changes were observed in blood glucose values of animals ingested with hexane as well as chloroform extracts during whole 6 h screening period.

Table 2 also shows that, the oral administration of water extract of fenugreek seeds significantly restricted the increment of blood glucose after overloading of outsource glucose in rats and this effect was more prominent than other extracts. Ethanol extract also significantly inhibited the rise of blood glucose in these rats, but effect was moderate as compared to others. No significant changes were observed in the extracts of methanol, hexane and chloroform.

Table 1: Phytoconstituents present in various extracts of fenugreek seeds

Phyto-constituents (%)	Water extract	Ethanol extract	Methanol extract	Hexane extract	Chloroform extract
Alkaloids	0.59±0.13^{a}	0.44±0.12^{a}	5.56±0.19^{b}	7.34±0.15^{c}	8.49±0.11^{d}
Phenols	0.47±0.08^{a}	0.46±0.06^{a}	0.39±0.10^{a}	0.55±0.13^{a}	0.43±0.09^{a}
Tannins	21.21±0.24^{a}	11.43±0.54^{b}	5.12±1.30^{c}	2.32±1.32^{d}	1.43±0.56^{d}
Falvonoids	1.42±0.36^{a}	1.22±0.32^{a}	1.53±0.06^{a}	4.34±0.11^{b}	3.55±0.14^{c}
Saponin	6.77±0.76^{a}	1.57±0.54^{b}	1.43±0.46^{b}	0.43±0.54^{c}	0.77±0.33^{c}

Values are means±SD of three independent measurements of each extract, Values with different superscripts are significantly different at the level of $p<0.05$

Table 2: Hypoglycemic potential of various extracts of fenugreek seeds in normal and diabetic rats

Time (h)	Water extract	Ethanol extract	Methanol extract	Hexane extract	Chloroform extract
Normal rats					
0	123±9.4^{aA}	132±12.2^{aA}	129±9.7^{aA}	122±11.6^{aA}	119±9.4^{aA}
1	81±10.4^{aB}	101±11.5^{aB}	119±11.4^{bA}	125±11.4^{bA}	121±8.5^{bA}
2	72±14.2^{aB}	990±8.9^{bB}	112±10.5^{cB}	126±10.7^{cA}	117±11.3^{cA}
3	82±11.8^{aB}	103±8.1^{bB}	121±13.2^{cA}	122±10.2^{cA}	119±10.4^{cA}
4	99±10.2^{aB}	114±11.7^{bA}	124±10.4^{bA}	129±9.7^{bA}	128±8.9^{bA}
5	109±9.8^{aA}	129±12.4^{bA}	128±10.3^{bA}	124±10.5^{bA}	124±11.4^{bA}
6	131±14.2^{aA}	124±14.3^{aA}	130±9.8^{aA}	123±11.2^{aA}	128±12.7^{aA}
Hyperglycemic rats					
0	131±13.2^{aA}	129±12.5^{aA}	121±9.9^{aA}	131±12.3^{aA}	134±9.5^{aA}
1	158±10.8^{aB}	172±10.2^{bB}	233±20.2^{cB}	259±18.2^{cB}	277±18.4^{cB}
2	126±9.7^{aA}	155±14.2^{bC}	171±17.3^{cC}	191±18.9^{cC}	169±12.7^{cC}
3	120±13.1^{aA}	126±12.5^{bA}	155±12.9^{bC}	143±14.2^{cA}	167±15.5^{cC}
4	131±17.2^{aA}	128±11.3^{bA}	133±10.8^{bA}	129±12.8^{bA}	138±12.9^{bA}
5	121±12.8^{aA}	132±13.5^{aA}	122±9.1^{aA}	134±15.3^{aA}	123±19.4^{aA}
6	125±14.6^{aA}	128±10.1^{aA}	128±11.4^{aA}	130±17.1^{aA}	128±16.8^{aA}

Values are means±SD of six animals in each group; ^{a,b,c}: Values with different superscripts in a row are significantly different at the level of $p<0.05$, ^{A,B,C}: Values with different superscripts in a column are significantly different at the level of $p<0.05$

Table 3: Complementary hypoglycemic potential of water extract of fenugreek seeds and glimepiride in normal and diabetic rats

	Time (h)						
Groups	0	1	2	3	4	5	6
Normal rats (mg kg^{-1} b.wt.)							
Water extract							
200	126±9.3^{aA}	86.0±9.4^{bA}	74.0±10.4^{bA}	112±11.3^{bA}	112±10.3^{bA}	119±10.5^{aA}	128±11.2^{aA}
100	122±7.9^{aA}	109.0±8.4^{bB}	119.0±17.2^{bB}	122±10.2^{bB}	128±9.5^{aA}	121±9.4^{aA}	124±9.8^{aA}
50	129±11.3^{aA}	109.0±9.5^{bB}	118.0±10.1^{bB}	119±9.80^{bB}	121±10.9^{aA}	128±10.2^{aA}	127±10.3^{aA}
Glimepiride							
20	119±10.5^{aA}	78.0±9.5^{bA}	88.0±9.4^{bA}	103±9.7^{bA}	118±12.0^{aA}	121±11.8^{aA}	123±12.4^{aA}
10	118±11.7^{aA}	89.0±10.3^{bA}	97.0±10.2^{bB}	109±10.9^{aA}	113±14.1^{aA}	119±10.3^{aA}	117±10.2^{aA}
5	127±12.3^{aA}	103.0±11.1^{bB}	113.0±9.4^{bB}	115±10.4^{bA}	122±10.2^{aA}	131±9.4^{aA}	126±13.3^{aA}
Water extract+glimepiride							
200+20	125±11.8^{aA}	65.0±9.4^{bA}	34.0±10.2^{cA}	ND	ND	ND	ND
200+10	121±9.7^{aA}	72.0±10.1^{bA}	35.0±11.9^{cA}	ND	ND	ND	ND
200+5	118±9.8^{aA}	78.0±10.9^{bA}	41.0±12.4^{cA}	450±11.2^{c}	570±10.8^{c}	890±7.3^{b}	105±10.4^{aA}
Hyperglycemic rats (mg kg^{-1} b.wt.)							
Water extract							
200	132±13.7^{aA}	159.0±6.3^{bA}	137.0±7.7^{aA}	128±8.6^{aA}	124±6.9^{aA}	113±7.8^{aA}	129±10.8^{aA}
100	131±12.1^{aA}	167.0±7.4^{bB}	147.0±11.4^{bA}	129±9.9^{aA}	129±8.3^{aA}	118±10.2^{aA}	121±11.3^{aA}
50	127±8.7^{aA}	192.0±10.9^{bC}	176.0±12.2^{cB}	129±11.8^{aA}	128±8.9^{aA}	122±9.9^{aA}	130±7.1^{aA}
Glimepiride							
20	138±8.5^{aA}	172.0±6.2^{bA}	161.0±6.3^{cA}	142±6.9^{aA}	128±7.3^{aA}	123±11.8^{aA}	129±9.8^{aA}
10	121±9.2^{aA}	209.0±9.7^{bB}	169.0±9.2^{cA}	143±8.7^{dB}	129±8.1^{aA}	134±10.4^{aA}	124±5.4^{aA}
5	129±13.4^{aA}	234.0±9.7^{bC}	168.0±6.8^{cA}	134±6.8^{aA}	124±6.4^{aA}	144±8.2^{aA}	133±8.5^{aA}
Water extract+Glimepiride							
200+20	119±9.0^{aA}	101.0±8.4^{bA}	111.0±7.6^{aA}	123±8.8^{aA}	124±9.2^{aA}	118±9.4^{aA}	132±9.4^{aA}
200+10	122±7.9^{aA}	119.0±9.1^{aA}	125.0±10.9^{aA}	132±8.2^{aA}	117±5.4^{aA}	121±6.2^{aA}	128±8.3^{aA}
200+5	139±6.4^{aA}	142.0±9.8^{aA}	132.0±12.3^{aA}	133±6.4^{aA}	143±8.8^{aA}	129±5.9^{aA}	132±7.7^{aA}

Values are means±SD of six animals in each group; ^{a,b,c,d}: Values with different superscripts in a row are significantly different at the level of $p<0.05$; ^{A,B,C}: Values with different superscripts in a column (dose dependent for a particular group) are significantly different at the level of $p<0.05$; ND = Not Determined

Complementary Hypoglycemic and Anti-Hyperglycemic Activity of Water Extract with Glimepiride

The results in Table 3 shows that hypoglycemic and anti-hyperglycemic potential of water extract of fenugreek seeds were dose dependent and decreased with the dose. Similarly dose dependent hypoglycemic and anti-hyperglycemic effects of glimepiride also noted. Thereby, 200 mg kg^{-1} b.wt. of water extract was selected for further complimentary activity with different doses of glimepiride. Oral administration of water extract (200 mg kg^{-1} b.wt.) along with 20 and 10 mg kg^{-1} b.wt. of glimepiride developed chronic hyperglycemia after 2.5-3 h and animals were felt uncomfortable and shocked. Animals were saved by injecting (i.p.) 2 g kg^{-1} b.wt. of glucose solution and discontinued to blood glucose screening. However, the blood glucose levels were significantly decreased in animals ingested with 200 mg kg^{-1} b.wt. of water extract and 5 mg kg^{-1} b.wt. of glimepiride, but not developed chronic hypoglycemia and blood glucose returned towards increasing fashion after 4 h.

It may also be shown in Table 3 that, oral administration of 200 and 20 mg kg^{-1} b.wt. of glimepiride fully restricted to increase the blood glucose after oral load of glucose solution also and slightly decreased from normal level after 1 h of screening period at this dose. The dose of 10 mg kg^{-1} b.wt. of glimepiride with water extract also not allowed to increase blood glucose significantly during whole screening period (6 h). Slight increase in blood glucose was observed in animals administered with the dose of 200 mg kg^{-1} water extract and 5 mg kg^{-1} of glimepiride after 2 h, which was significantly lower than those control animals.

DISCUSSION

In present study, the water extract of fenugreek seeds exhibited highest hypoglycemic and anti-hyperglycemic potential among five extracts and shown good complementary activity with glimepiride. Hyperglycemia is a chronic state which needs a deeper attention to control, because chronic hyperglycemia is a causative factor of several microvascular and macrovascular complications of diabetes (Brindisi *et al*., 2006). Indeed, the control of blood glucose levels is a hallmark in the management of diabetes mellitus, various therapeutic strategies have been applied to control blood glucose levels in which use of medicinal plants is a significantly recognized one (Grover *et al*., 2002). After a thorough reviewing the literature on anti-diabetic effects of fenugreek, it has been found that various studies reported various combinations/extracts of fenugreek seeds in different diabetic models, but it has not been clear which is the best and safer to be used for human consumption. Thereby present study was conducted to find out the safer and best hypoglycemic extract of fenugreek seeds. Water extract was found best for its hypoglycemic and anti-hyperglycemic potential among five extracts (ethanol, methanol, hexane and chloroform) of fenugreek seeds, which is safer than other extracts and can be used directly for human consumption. The exact reason behind the highest hypoglycemic and anti-hyperglycemic activity of water extract is not known, but on the basis of the results of present study it may speculated that this activity was might be due higher content of tannins and saponins in water extract than those of other extracts (Table 1). Tannins and saponins are water soluble components have been reported for hypoglycemic potential (Diatewa *et al*., 2004; Suba *et al*., 2004). Moreover, in present study the combination of water extract with standard drug; glimepiride was also investigated to find out how much dose of glimepiride can be reduced by combining water extract of fenugreek seeds. This part of this study was very interested that, the combination of water extract significantly reduced the dose of glimepiride (from 20 to 5 mg kg^{-1} b.wt.) in glucose over-loaded animals and it was safer in normal animals also, whilst combination with higher doses (20 and 10 mg kg^{-1} b.wt.) were chronic to produce hypoglycemia in normal rats. On the basis of these results it may be speculated that water extract of fenugreek seed had similar activity as glimepiride as an insulin secretor during glucose overloaded animals. The synergistic effect of water extract with glimepiride supports it as an insulin secretor activity, but in present study insulin levels were not estimated which needs further study.

In conclusion, it may suggest that the combination of water extract of fenugreek seeds may play an important role to reduce the blood glucose levels in chronic diabetic conditions, but higher dose may cause hypoglycemic shock in normal or pre-diabetic state. Moreover, further study is required to isolation, purification and characterization of active components from the water extract which may pave a good independent and/ or complementary regimen for the treatment of diabetes mellitus.

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